Real-time Infectivity Monitoring for Measles Vaccine Production in Vero Cells Using Laser Force Cytology™

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Introduction

Vaccination to prevent infectious diseases is one of the most effective tools for improving public health and mitigating the effects of large-scale disease outbreaks such as the COVID-19 pandemic[1]. In order to enable widespread adoption of vaccines, they must be manufactured at scale in a timely and cost-effective manner. In general, however, the production of vaccines, including live virus vaccines (LVVs) is significantly more complex than other biological production systems such as monoclonal antibodies and thus requires additional time and resources[2-4]. For example, the potency of a live virus vaccine (LVV) is generally the most critical measurement of efficacy, but existing methods to measure the potency of viruses, including the plaque and the TCID50 assays, have very long lead times and cannot provide real time information on product quality during large scale manufacturing[5, 6].

LumaCyte's Laser Force Cytology™ (LFC) Radiance® instrument uses a combination of optical pressure and microfluidics to interrogate individual cells. LFC measures the intrinsic biophysical and biochemical properties of single cells and has the ability to monitor the production of virus in minutes, as opposed to waiting days to weeks for a typical infectivity assay, thus allowing for rapid process optimization, better process knowledge, and increased product yield by more precisely timing the harvest and adjusting the purification process based on yield as applicable.

Here we present the application of LFC as a valuable process analytical tool (PAT) to measure potency of LVVs in upstream biomanufacturing processes with the goal of improving the speed, efficiency and quality of vaccine development and manufacturing.

Experimental Results

Figure 1 provides a detail of the sample harvest process for samples run on Radiance®, LumaCyte's LFC instrument. Specifically, Vero cells were seeded onto microcarriers and then incubated for several days to allow for the cells to become confluent, at which point they were infected with measles virus (MV). At each timepoint post infection,

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a sample was withdrawn from the bioreactor and separated into two fractions. The first contains the microcarriers with cells attached, and the second contains the supernatant as well as any cells that have separated from the microcarriers throughout the production process. The microcarrier fraction is then processed to detach the cells as described in the Methods section. From there, the separate microcarrier and supernatant samples are centrifuged and then resuspended in LumaCyte Stabilization Fluid at a concentration of approximately 750,000 cells/mL in a volume of 200 μL (150,000 cells total) for analysis with Radiance® as separate samples.

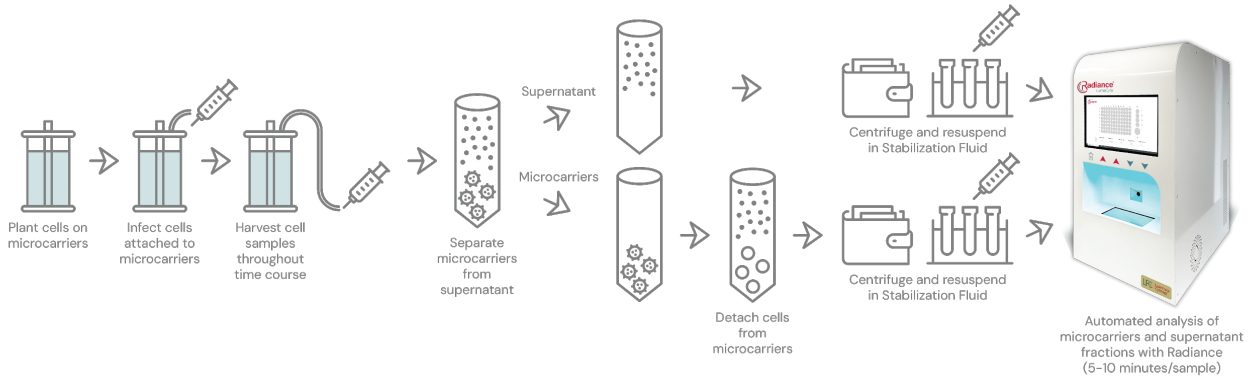

Figure 1. Microcarrier Sampling Workflow for Analysis with Radiance®. Samples are harvested throughout the production time course and separated into distinct supernatant and microcarriers fractions prior to analysis with LumaCyte's Radiance® instrument.

As demonstrated previously[7, 8], LFC can be used to monitor the infection time course by identifying one or more parameters measured by Radiance® that can be correlated with the viral infectivity, referred to as the Infection Metric. In the case of numerous viruses[7, 9], the optical force and thus velocity changes with infection and can be incorporated into the Infection Metric. As each cell flows through the microfluidic channel, the laser within Radiance® exerts an optical force on the cell, reducing its velocity. This change in velocity is due to the transfer of momentum from the photons in the laser as they scatter and refract through the cell and is a function of the biophysical and biochemical state of the cell. Therefore, an initial starting point for determining the cellular changes that occur throughout the infection time course is the Size vs Velocity scatter plot. Scatter plot data from a representative bioreactor experiment is presented in **Figure 2**. Specifically, each graph compares the uninfected cells (**green**) detached from the microcarriers just prior to infection to the supernatant sample collected at the time indicated on the graph (**red**), starting at 3 days post infection. In general, minimal signs of infection are seen prior to day 3 post infection across multiple measurements including LFC and other potency assays.

As can be seen in **Figure 2**, as the infection progresses from 3 to 6 days post infection, the infected supernatant population shifts substantially away from the uninfected population, overall decreasing in velocity, reflecting an increase in the optical force index (OFI) or size normalized velocity. OFI was developed to help normalize any size changes that occur with infection and help describe the effects on optical force independent of cell size[7]. While a

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decrease in velocity represents an increase in optical force, the velocity component of the OFI parameter is the difference between the fluid velocity and the cell velocity.

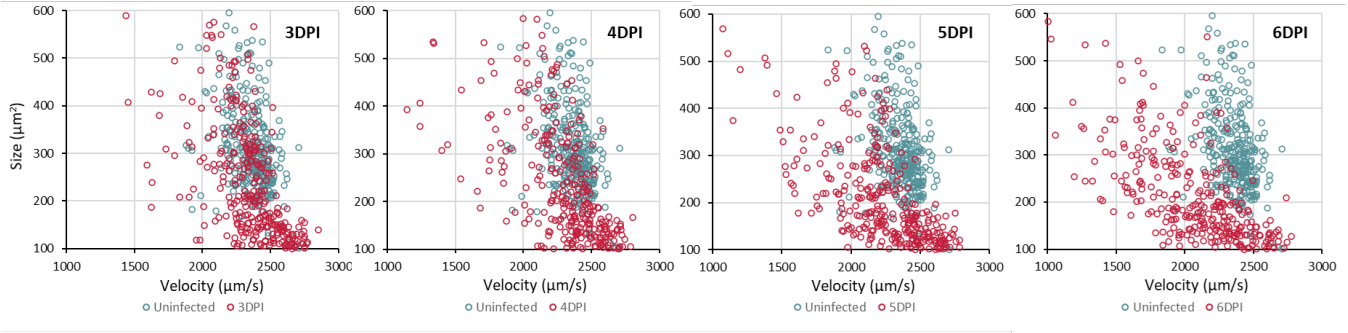

Figure 2. Radiance Velocity vs Size Scatter Plots. Comparison of the velocity (proportional to the optical force) and area-based size of uninfected cells (green) detached from the microcarriers just prior to infection to supernatant samples collected at the time indicated on the graph (red), starting with 3 days post infection. Each point on the plot represents a single cell within the respective population.

Thus, an increase in OFI represents an increase in the optical force exerted on each cell. OFI histograms for the supernatant fraction of the same data set are presented in **Figure 3** and show a progression of increasing optical force as the infection progresses. Similar to gating in flow cytometry[10], the percentage of a population above a threshold value can be used to help describe the population. In this case, the empirically determined threshold of 55 (s^{-1}) was selected and is shown on each of the histogram plots. As shown in **Figure 3,** the percentage of cells above 55 increases from 23.6% at 3 days post infection to 71.5% at 6 days post infection, demonstrating an increase in the optical force as the infection progresses. Based on this behavior, this OFI threshold value was selected as the Infection Metric.

Once identified, the Infection Metric can be used to track the progress and kinetics of an infection. However, to use LFC to calculate other biological parameters, such as LVV potency, a quantitative correlation must be developed between the biological parameter of interest and the Infection Metric.

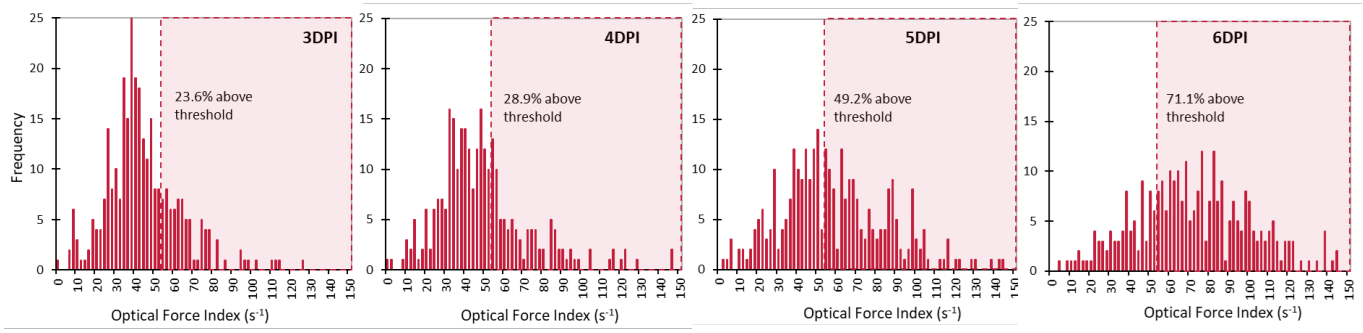

Figure 3. Radiance® Optical Force Histograms. Histograms showing the optical force index (OFI) population distribution from supernatant cell samples harvested from 3-6 days post infection as indicated. The percentage of the cell population with an OFI > 55 s^{-1} is shown on each plot and highlighted in the red box.

In this case, the biological parameter used was estimated potency as measured by flow virometry. Although this is a physical rather than infectious titer measurement, this has been used as a correlation to the TCID50 based potency assay for MV during production. However, should the ratio of total to infectious particles change due to some unforeseen or undetected process perturbation, a cell-based PAT such as LFC capable of potentially detecting this change would be required.

Because LFC makes measurements on a per-cell basis and the estimated potency measurement in this case is a per-volume measurement, the cell count must also be considered in order to accurately correlate the LFC data with the viral particle measurement. Cell counts, both for the microcarriers and supernatant fractions, are shown in **Figure 4A** for each of the three experiments. As illustrated in the figure, no significant detachment of cells from the microcarriers into the supernatant is seen until 3 days post infection, after which point the percentage of detached cells increases significantly.

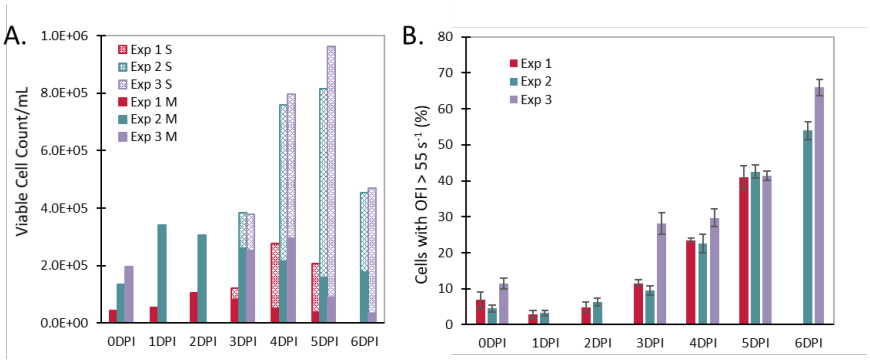

Figure 4. Viable Cell Count and Radiance® Optical Force Index (OFI) data from Bioreactor Samples. A. Viable cell counts for the supernatant (S) and microcarriers (M) fraction at each time point. B. The percentage of the overall cell population with an OFI greater than 55 s^{-1} at each time point (infection metric). This value accounts for the percentage of cells in both the S and M fractions. Results are shown for each assay for bioreactor runs from 3 separate experiments.

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Based on the cell count of both the microcarriers and supernatant fraction as well as the number of virus particles per mL, the number of virus particles per viable cell can be calculated and is shown on the Y axis of **Figure 5**. As Radiance measures the microcarriers and supernatant fractions separately, the infection metric also needs to be adjusted based on the overall percentage of the cell population represented by the microcarriers and supernatant fraction, respectively. Specifically, the population combined OFI metric was calculated based on the following equation:

$$Combined\ OFI\ Metric\ (\%) = \frac{\frac{cells}{mL}_{M}}{\frac{cells}{mL}_{Total}} \times \%\ cells\ OFI > 55_{M} + \frac{\frac{cells}{mL}_{S}}{\frac{cells}{mL}_{Total}} \times \%cells\ OFI > 55_{S}$$

The combined OFI metric taking into account both the microcarriers and supernatant fraction is presented in **Figure 4B** and is the x-axis parameter of the scatter plot in **Figure 5**, which shows a strong correlation with the total virus particles per viable cell. This demonstrates the capability to dynamically monitor the estimated yield of virus using the Radiance® data, allowing for a rapid measurement of potency that could be used to understand, monitor, and potentially adjust the production process in minutes as opposed to hours or days for typical potency measurements.

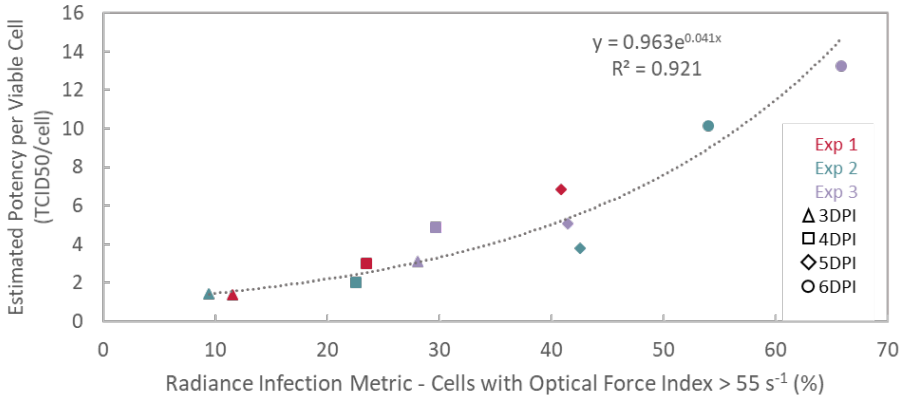

Figure 5. Correlation between Radiance® Infection Metric and Estimated Potency. Population-wide correlation between Radiance® data and estimated potency on a per viable cell basis as measured by the total virus particles. Each point represents the timepoint and experiment indicated on the plot.

Discussion

The recent COVID-19 pandemic has highlighted the need to improve the speed of the development and manufacturing of vaccines of all types. LVVs have an established history of safety and efficacy, but their development has been comparatively slow, often taking decades or more[2-4]. One factor contributing to the slow

pace of development is the challenge associated with accurately characterizing the potency of LVVs, which historically has been achieved using infectivity assays such as the plaque or TCID50 assay[5, 6]. These assays are labor intensive, highly variable, and often subjective[11, 12], making them in general a rate-limiting step in the vaccine development and manufacturing process. In addition, these assays are unable to provide the rapid results required for use as a PAT, which would have numerous benefits in terms of ensuring the quality and maximizing the quantity of LVVs.

Here we demonstrate the use of LFC as a PAT for monitoring vaccine potency using MV produced by Vero cells growing on microcarriers. The infection was tracked throughout multiple independent bioreactor runs using cellular changes measured by LFC in both the supernatant and microcarriers cell fractions. Importantly, by combining the data from both fractions, a strong correlation was developed between the percentage of high optical force cells (OFI > 55 s^{-1}) and the estimated potency, with an average absolute log10 difference of 0.07 between the measured and calculated values (**Table 1**). This demonstrates the capability for LFC to provide rapid and accurate feedback on potency measurements to improve the speed of process optimization by reducing time to result as well as provide ongoing feedback to inform the manufacturing and potentially downstream purification processes by optimizing the purification steps based on the potency readout. LFC provides these results without the requirement for antibodies or other labels and with an automated and quantitative readout. Using LumaCyte's ReportR™ software, the potency calculation based on the correlation shown in **Figure 5** can be automatically performed and a report sent to the end user, providing increased speed and automation. Both the instrument and ReportR™ are 21 CFR Part 11 compliant, providing additional functionality from a regulatory compliance perspective.

Experiment	Sample (h)	Radiance® Infection Metric (% OFI>55 s^{-1})	Estimated Potency (TCID50/viable cell)	Calculated Potency (TCID50/viable cell)	Log_{10} Difference
Exp 1	72	11.53	1.36	1.55	0.056
	96	23.52	2.97	2.54	-0.068
	120	40.92	6.81	5.22	-0.116
Exp 2	72	9.49	1.38	1.42	0.015
	96	22.60	1.98	2.45	0.093
	120	42.57	3.79	5.59	0.169
	144	54.02	10.11	8.97	-0.052
Exp 3	72	28.12	3.06	3.08	0.002
	96	29.69	4.85	3.28	-0.169
	120	41.48	5.04	5.34	0.025
	144	65.92	13.22	14.67	0.045
				Absolute Average	**0.074**

Table 1. Estimated and Calculated Potency Values for MV Bioreactor Samples. Table detailing the combined Radiance® Infection Metric, Estimated Potency, and Calculated Potency based on the correlation between the LFC data and estimated potency. Finally, the average log10 difference between the two for each sample timepoint as well as the absolute average for all points are given in the final column.

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In summary, utilizing LFC as a rapid (PAT) for potency monitoring and as an analytical assay for measuring infectious titer streamlines the research, development, and manufacturing timeline for LVVs and other virus-based vaccines, including protein subunit vaccines produced in Sf9 cells via baculovirus or adenovirus-based viral vector vaccines. This rapid, cell-based infectivity assessment has the potential to enhance the entire vaccine development lifecycle, from R&D to clinical trials and manufacturing, ultimately reducing costs and time associated with LVVs and other vaccines.

Cell Culture, Infection, and Harvest

Vero cells (ATCC CCL-81) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum at 37°C in a humidified incubator with 5% CO2 prior to seeding onto microcarriers in a bioreactor. After a period of growth, cells were infected with MV at a defined MOI and monitored for up to 6 days post infection. Samples containing both microcarriers and supernatant were withdrawn daily for cell count, viability, viral particle, and Radiance analysis. For Radiance® analysis, microcarriers with attached cells were separated from any free-floating cells in the supernatant by allowing the microcarriers to settle for about 1 minute and then transferring the supernatant to a separate tube. In order to detach the cells from the microcarriers, samples were rinsed with two separate volumes of TrypLE (Thermo Fisher Scientific) prior to a 10-minute incubation time in TrypLE at 37° C to detach the cells. Following this incubation, samples were vigorously mixed to help detach the cells from the microcarriers. Both the microcarriers and supernatant fractions (still separate) were then filtered using a 37 m cell strainer to remove any remaining microcarriers and then centrifuged for 5 minutes at a speed 200g prior to resuspension LumaCyte Stabilization Fluid with 0.5% paraformaldehyde (PFA) to a final concentration of approximately 750,000 cells/mL. Cell samples (200 ?L) were then loaded into the 96-well plate for analysis with a Radiance® instrument.

Acknowledgement

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